J. Clin. Chem. Clin. Biochem.
Vol. 22, 1984, pp. 467–471

Placental Alkaline Phosphatase in Tumour Tissue and Serum

By *Ellen Mössner, G. Pfleiderer*

Institut für Organische Chemie, Biochemie und Isotopenforschung der Universität Stuttgart and

K. D. Dittel

Chirurgische Abteilung des Marienhospitals, Stuttgart

(Received December 23, 1983/March 27, 1984)

Summary: Using immunochemical techniques, alkaline phosphatase isoenzymes were determined in tissue samples of breast carcinomas and carcinomas of the gastrointestinal tract. In breast carcinomas only 19% of the patients expressed significant placental alkaline phosphatase activity, compared with 78% in gastrointestinal tumours. The intestinal isoenzyme was found in 50% of the breast carcinomas and in nearly all of the other examined tissues. The two isoenzymes usually represent 1% of the total alkaline phosphatase activity, but in a few cases they may constitute between 10 and 90%. In the serum of the patients under examination, elevated total alkaline phosphatase activity was found in only 7%, and elevated placental alkaline phosphatase in 6% of the cases. No cases of elevated serum intestinal alkaline phosphatase were found. We therefore consider that serum placental alkaline phosphatase is a poor tumour marker for a general screening.

Placentare alkalische Phosphatase in Tumorgewebe und Serum

Zusammenfassung: In Gewebeproben von Mamma-Carcinomen als auch Carcinomen des Gastrointestinaltraktes wurden mit empfindlichen immunologischen Methoden die Isoenzyme der alkalischen Phosphatase bestimmt. Bei Brustkrebs zeigten nur 19% der Patienten signifikante Aktivität der placentaren alkalischen Phosphatase, verglichen mit 78% bei gastrointestinalen Tumoren. Das intestinale Isoenzym wurde in 50% bei Brustcarcinom und in nahezu allen anderen malignen Geweben gefunden.

Meist betrug die katalytische Aktivität der beiden Isoenzyme unter 1% der gesamten katalytischen Aktivität der alkalischen Phosphatase, in wenigen Fällen zwischen 10 und 90%. Im Serum der untersuchten Patienten wurde nur in 7% der Fälle überhaupt erhöhte katalytische Aktivität der gesamten alkalischen Phosphatase beobachtet, nur in 6% der Fälle placentare alkalische Phosphatase und in keinem der Fälle erhöhte intestinale alkalische Phosphatase gefunden. Wir glauben daher, daß die placentare alkalische Phosphatase für ein generelles Screening ein unbedeutender Tumormarker ist.

Introduction

Since its discovery as the *Regan* isoenzyme, serum placental alkaline phosphatase in a non-pregnant person has been considered to be a product of oncodevelopmental gene expression, and regarded as tumour marker (1). Owing to the use of different assay methods and the immunological cross-reactivity of the placental and intestinal alkaline phosphatase, controversy has arisen regarding the sensitivity of the placental alkaline phosphatase as a tumour marker.

With highly sensitive tests, low levels of placental alkaline phosphatase have been found in the sera of some healthy persons (2, 3), and slightly increased levels in smokers (4, 5).

Also, placental alkaline phosphatase has been found as a minor component in the lung (6), testis (7), ovary (8) and cervix (9, 10, 6). Thus, in the normal adult, placental alkaline phosphatase might be produced by a slight expression of developmental genes, which is increased in cancer cells.

We developed a sensitive and easily performed immunoassay for the quantitative determination of placental alkaline phosphatase and also for intestinal alkaline phosphatase in serum and tissue extracts. In a screening test of 174 sera from treated cancer patients, we found elevated placental alkaline phosphatase values in only 10% of the samples (11). This unsatisfactory result led us to the quantitative determination of placental alkaline phosphatase in tumour tissue and its possible appearance in the sera of the patients prior to therapy and surgery.

Materials and Methods

Biological materials

Tissue samples were obtained at operation. Sera gained prior to surgery and the tissue were stored no longer than 24 h at 4 °C or at −20 °C until used.

The tissue (samples between 0.3 and 8.0 g) was homogenized in an ultra turrax homogenizer with 10 mmol/l Tris/HCl pH 7.5, containing 2 mmol/l $MgCl_2$ and 0.025 mmol/l $ZnCl_2$ (1:3 w/v), *n*-butanol was added until a concentration of 30% was reached. After stirring at 4 °C for 48 h the homogenate was centrifuged (40000 *g*) and the aqueous layer, containing the enzyme, was dialysed against the homogenization buffer and used for analysis.

Antibodies

The IgG fraction of sheep anti-human intestinal alkaline phosphatase serum (Merck, Darmstadt) was isolated by 2 × precipitation in 50% saturated $(NH_4)_2SO_4$ solution followed by dialysis against 10 mmol/l Tris/HCl buffer, pH 7.5. Anti-human placental alkaline phosphatase antibodies were raised in rabbits (titer: 25 U placental alkaline phosphatase precipitated per mg IgG). The IgG fraction was isolated by affinity chromatography over protein A-sepharose Cl 4 B: To a 8 × 1 cm column, equilibrated with 10 mmol/l Tris/HCl buffer, pH 7.5, 4 ml of serum was applied. After 1 h incubation, protein was eluted with the equilibration buffer. The bound IgG-fraction was eluted with 0.1 mol/l glycine/HCl buffer, pH 3.0. Fractions (5 ml) were collected on ice and neutralised with $(NH_4)_2CO_3$. The total IgG fraction was concentrated by ultrafiltration to the original serum volume, then dialysed against a 10 mmol/l Tris/HCl buffer, pH 7.5.

Intestine alkaline solid-phase direct immuno assay

Polystyrene tubes (50 × 7 mm, Greiner, Nürtingen) were coated with anti-intestinal alkaline phosphatase IgG by incubating them with 1 ml of antibody solution (10 mg/l in 10 mmol/l phosphate buffer, pH 7.0) for 2 days at room temperature. Non-absorbed antibodies were removed by suction and the tubes were washed three times with phosphate buffered saline (9 g NaCl, 0.27 g KH_2PO_4, 1.43 g $Na_2HPO_4 \cdot H_2O$ ad 100 ml H_2O, pH 7.4). Tissue extracts (900 µl) or serum (100 µl) were pipetted into the tubes together with 800 µl of 10 mmol/l Tris/HCl buffer (pH 7.5, containing 2 mmol/l $MgCl_2$ and 0.025 mmol/l $ZnCl_2$ and 3% polyethylene glycol 6000) and the mixture was incubated for 1 h at 37 °C followed by 24 h in the cold. After removing the solution by aspiration and washing three times with 1 ml phosphate buffered saline, containing 3% polyethylene glycol 6000, the enzymatic activity bound to the antibody-coated tube, was determined by incubating the tubes at 25 °C in 900 µl 1 mol/l diethanolamine/HCl, pH 9.8 containing 0.5 mmol/l $MgCl_2$, 10 mmol/l *p*-nitrophenyl phosphate as substrate and 5 mmol/l *L*-leucyl-glycyl-glycine to inhibit placental alkaline phosphatase. At a concentration of 5 mmol/l this inhibitor inactivates 90% of the placental isoenzyme, but only 15% of the intestinal alkaline phosphatase. As only a part of the placental isoenzyme is bound to the anti-intestinal alkaline phosphatase coated tubes, a good discrimination is possible. After 60 min the reaction was stopped by addition of 100 µl 5 mol/l NaOH, containing 10 mmol/l EDTA, and the absorbance was measured at 405 nm against a buffer blank. The enzymatic activity was referred to standard samples with known activity of the isoenzyme in 100 µl serum or incubation buffer.

Placental alkaline solid-phase direct immunoassay

One ml of solution, containing 8 mg anti-placenta alkaline phosphatase IgG in 3 mmol/l phosphate buffer, pH 6.3, was added to an immuno-bead suspension (200 mg in 20 ml buffer, Bio-Rad Laboratories, Richmond/CA) and incubated for 1 h at room temperature. To this 40 mg 1-ethyl(-dimethyl-amino propyl)-carbodiimide hydrochloride (EDAC) was added, mixed vigorously and stored at 4 °C for 3 h. The mixture was equally divided amongst four 25 ml centrifuge tubes and each aliquot was suspended in phosphate buffered saline to make 25 ml. The tubes were centrifuged at 1000 *g* for 10 min. The supernatant was decanted, the pellet washed three times by suspension in phosphate buffered saline and centrifugation, followed by 2 washes in 1.4 mmol/l NaCl/phosphate buffered saline and 2 washes in phosphate buffered saline. The mixture was resupended in phosphate buffered saline and allowed to stand on ice for 3 h in order to renature the antibody. After centrifugation, the pellet was resuspended in 20 ml 10 mmol/l Tris/HCl buffer, containing 2 mmol/l $MgCl_2$, 0.025 mmol/l $ZnCl_2$ and 0.02% NaN_3. Protein determination in the supernatant revealed that, on average, 35% of the IgG fraction is coupled to the immuno-beads.

The bead suspension (50 µl) (possessing covalently bound anti-placental alkaline phosphatase) was added to the serum or tissue extract. After 1 hour incubation at 37 °C and 2 washing and centrifugation steps (phosphate buffered saline, containing 3% polyethylene glycol 6000, 10 min 1000 *g*) the pellet was resuspended in 1 ml 10 mmol/l Tris/HCl buffer, pH 7.5, containing 2 mmol/l $MgCl_2$, 0.025 mmol/l $ZnCl_2$ and 3% polyethylene glycol 6000 and heated for 90 min at 65 °C to inactivate the partially bound intestinal isoenzyme. Placental alkaline phosphatase remains unaffected by this heating step, retaining 100% of its catalytic activity, while the intestinal isoenzyme is completely inactivated. With shorter heating periods, up to 10% of the intestinal alkaline phosphatase might be still active. After centrifugation for 10 min at 1000 *g*, the pellet was resuspended in 900 µl 1 mol/l diethanolamine/HCl pH 9.8 containing 0.5 mmol/l $MgCl_2$ and 10 mmol/l *p*-nitrophenyl phosphate. The catalytic activity was determined as described for intestinal alkaline phosphatase.

Results

Characterization of the solid phase direct immunoassays

Calibration curves with purified human intestinal alkaline phosphatase in the catalytic concentration range 0.2−3 U/l were linear, and the analytical recovery was about 70% when the enzyme was assayed in buffer, and 40−50% when the enzyme was assayed in serum. Samples with a catalytic concentration >3 U/l were diluted in buffer. The intra-as-

say coefficient of variation (determined in 100 µl human serum, 800 µl buffer with purified enzyme added) was 5.7% (mean 2.44 U/l, n = 10).

This solid-phase assay, using antibody-coated tubes, could also be applied to the determination of placental alkaline phosphatase in tissue extracts. On the other hand, the low concentration of serum placental alkaline phosphatase might lead to poor analytical recoveries, using 1 ml serum for the determination.

The immuno-beads assay for placental alkaline phosphatase determination (catalytic concentration range = 0.2–3 U/l) showed an analytical recovery of 60–80% for enzyme standards in buffer or in 1 ml serum. The intra-assay coefficient of variation (determined in 1 ml human serum with purified enzyme added) was 3.3% (mean 0.3 U/l, n= 10) and 5.0% (mean 2.2 U/l, n= 10).

Alkaline phosphatase isoenzyme in cancerous tissues and sera of carcinomatoid patients

We determined total alkaline phosphatase catalytic concentration as well as the placental and intestinal isoenzyme in 170 sera of carcinomatoid patients prior to surgery or therapy, and in 111 tissue samples obtained by surgery. The results are summarized in table 1 and 2. Serum intestinal and placental alkaline phosphatase catalytic concentrations up to 8 U/l or 0.4 U/l were regarded as negative; the detection limit for tissue extracts was 1 mU/g tissue.

Placental alkaline phosphatase was found in 20% of the breast carcinomas and in 75% of the gastrointestinal carcinomatoid tissues. In most cases, however, only a few mU/g tissue placental alkaline phosphatase was detected (less than about 1% of the total alkaline phosphatase activity).

Tab. 1. Alkaline phosphatase isoenzymes in cancerous and benign tissue extracts.

Tissue	n	Total alkaline phosphatase mU/g[1]) (mean value)	Placental alkaline phosphatase mU/g[1]) (mean value)	n_r[2])	n−[3])	Intestinal alkaline phosphatase mU/g[1])	n_r[2])	n−[3])
Breast fibroma	1	200	0	0	1	1	1	0
Breast carcinoma	36	200–5200 (1007)	0–36	5	31	0–11	17	19
Breast carcinoma Bone metastasis	9	1500–6500 (20522)	0–2045 (228)	4	5	0–174 (25)	8	1
Stomach benign	4	1100–4200 (2000)	0	0	4	1–882 (234)	4	0
Stomach carcinoma	19	400–26300 (3813)	0–2000 (178)	13	6	0–1050 (130)	18	1
Sigma diverticulitis	3	500–11000 (733)	0–4 (2)	2	1	6–18 (13)	3	0
Sigma carcinoma	11	600–2000 (1265)	0–38 (8)	10	1	3–53 (24)	11	0
Colon carcinoma	12	500–11000	0–76	9	3	3–889	12	0
Rectum carcinoma	14	140–6100 (1310)	0–18 (2.5)	10	4	0–94 (21)	12	2
Bronchial carcinoma	1	95000	1	1	0	1	1	0
Bronchial carcinoma Bone metastasis	1	3700	1	1	0	4	1	0
Gall bladder carcinoma	2	1000–5200	0–4	1	1	0.2–21	2	0
Duodenal carcinoma	1	300	69	1	0	21	1	0
Kidney carcinoma	1	1600	2	1	0	35	1	0
Ovarial carcinoma	1	3700	104	1	0	22	1	0

[1]) mU alkaline phosphatase per gram tissue.
[2]) No. positive cases.
[3]) No. negative cases.

Tab. 2. Alkaline phosphatase isoenzymes in sera of cancer patients.

Diagnosis	n	Total alkaline phosphatase U/l (range)	No. of patients >190 U/l[1])	Intestinal alkaline phosphatase U/l (range)	No. of patients >8 U/l[2])	Placental alkaline phosphatase U/l (range)	No. of patients >0.4 U/l[3])
Breast carcinoma	62	(28–246)	2	(0–14.5)	2	(0 –0.7)	3
Stomach carcinoma	29	(30–356)	2	(0– 2)	0	(0 –1.0)	2
Rectum carcinoma	21	(30–293)	1	(0– 2)	0	(0 –0.3)	0
Colon carcinoma	20	(20–1337)	1	(0– 2)	0	(0 –0.3)	0
Sigma carcinoma	20	(30–250)	1	(0– 2)	0	(0 –1.6)	2
Seminoma	4	(163–241)	2	0	0	(0 –9.6)	3
Bronchial carcinoma	2	(89–93)	0	(0– 2)	0	(0.2–0.8)	1
Different carcinoma	12	(42–651)	3	(0– 2)	0	(0 –0.5)	1

[1]) Reference value 40–190 U/l (Merck, Klinisches Labor, see l.c. (17)).
[2]) Reference value 0– 8 U/l (see *Mössner* (11)).
[3]) Reference value 0– 0.4 U/l (see *Mössner* (11)).

This explains the poor positive results in sera: Placental alkaline phosphatase was detected in only 7% of the tested sera. In cases of carcinomatoid tissue with a very high placental alkaline phosphatase content (1000 mU/g tissue, e.g. in one bone metastasis of a breast carcinoma and one stomach carcinoma) placental alkaline phosphatase was also found in the sera.

No placental alkaline phosphatase was detected in benign stomach or breast tissue, but it was present in 2 out of 3 tested cases of sigma diverticulitis. Intestinal alkaline phosphatase was detected (a few mU/g tissue) in 50% of the breast carcinoma tissue and most bone metastases; as expected, it was also found in the intestinal tissue. The very variable intestinal alkaline phosphatase content of the stomach tissue, benign and cancerous, must be explained by the inclusion of duodenum tissue in the surgery samples.

Very high total alkaline phosphatase activity content (15–65 U/g tissue) was found in 3 bone metastases of breast carcinomas and in 2 stomach carcinomas. With the exception of one bone metastasis, the latter tissue samples had only a little intestinal alkaline phosphatase and no placental alkaline phosphatase catalytic activity.

Discussion

Our experiments do not confirm the literature findings, that in cancer patients total alkaline phosphatase catalytic activity is raised and placental alkaline phosphatase is found quite frequently (12). In our patient collective (cancer of the breast and digestive tract), prior to any therapy, serum placental alkaline phosphatase was found in only 7% of 170 tested samples, and raised total alkaline phosphatase catalytic activity occurred with similar low frequency.

Owing to the sensitivity of the immunoassays used, the placental isoenzyme was detected in various normal tissues and in a few normal sera, where it represented a low percentage of total alkaline phosphatase activity. We measured placental alkaline phosphatase in over 50% of the examined tumour tissues, but in most cases the enzyme appeared in such a low catalytic concentration that its release into serum by cell turnover would give undetectable serum levels; with even more sensitive tests it might be shown to be in the range found in normal serum. In accordance with literature data (13, 14) we found, in the few samples tested (see also l.c. (11)), a good correlation between seminoma patients and serum placental alkaline phosphatase. Most of the gastrointestinal tumours showed the placental isoenzyme in the tissue, but not in the sera. Especially in these cases the high intestinal alkaline phosphatase content of the intestinal mucosa compared with the low intestinal alkaline phosphatase in fasted individuals, which shows a dependence on blood grouping and secretory status (15), must be kept in mind. These factors have never been considered in relation to serum placental alkaline phosphatase, but high intestinal alkaline phosphatase catalytic activity has been measured in faeces (16). Thus, placental alkaline phosphatase expressed in intestinal tumours might also reflected in the faecal excretion of the enzyme.

No placental alkaline phosphatase was found in benign stomach tissue, but it was found in 60% of the examined stomach carcinomas and in 75% of intestinal carcinomas, and in sigma diverticulitis.

Demonstration of a low placental alkaline phosphatase catalytic activity is not necessarily related to cancerous tissue. Measured in sera, placental alkaline phosphatase must be regarded as an unsatisfactory tumour marker.

References

1. Fishman, M. H., Inglis, N. J., Green, S., Anstiss, C. C., Gosh, N. K., Reif, A. E., Rustigan, R., Krant, M. J. & Stolback, C. C. (1968) Nature *219*, 697–699.
2. Haije, W. G., Murwaldt, J. H., Talerman, A., Umpers, T. J., Baggerman, L., Teelew, A. H., van der Pompe, W. B. & van Driel, J. (1979) Int. J. Cancer *24*, 288.
3. Usategui-Gomez, M. (1974) Cancer Research *34*, 2544.
4. Tonih, S. E., Ortmeyer, A. E., Shindilman, J. E. & Sussman, H. H. (1983) Int. J. Cancer *31*, 51–53.
5. Maslow, W. C., Muensch, H. A., Azawa, F. & Schneider, A. S. (1983) Clin. Chem. *29*, 260–263.
6. Goldstein, D. J., Cramer, P. & Hillert, U. (1982) Z. Gastroenterol. *18*, 208–215.
7. Chang, C. H., Angellis, D. & Fishman, W. H. (1980) Cancer Res. *40*, 1506–1510.
8. Schnek, R. (1982) Diplomarbeit Stuttgart.
9. Malkin, A., Kellen, I. A. & Caplan, B. (1979) Carcino-Embryonic Proteins (F.-G. Lehmann, ed.) Vol. 2, 679, Elsevier, Amsterdam.
10. Nozawa, S., Ohta, H., Izumi, S., Hayashi, S., Tsutsui, F., Kurihara, S. & Watanabe, K. (1980) Acta Histochem. Cytochem. *13*, 521.
11. Mössner, E. & Pfleiderer, G. (1983) Clin. Biochem. *16*, 28–30.
12. Lehmann, F.-G. (1975) Klin. Wochenschrift *53*, 585–587.
13. Wahren, B., Homsen, P. A. & Stigbrand, T. (1979) Int. J. Cancer *24*, 749–753.
14. Lange, P. H., Milan, J. L., Stigbrand, T., Vessella, R. L., Ruoslahti, E. & Fishman, W. H. (1982) Cancer Res. *42*, 3244.
15. Langmann, M. J. S., Leuthorld, E., Robson, E. B., Harris, J., Luffman, J. E. & Harris, H. (1966) Nature *212*, 41–43.
16. Lehmann, F.-G., Cramer, P. & Hillert, U. (1980) Z. Gastroenterol. *18*, 208–215.
17. Schlebusch, H., Rick, W., Lang, H. & Knedel, M. (1974) Dtsch. Med. Wochenschr. *99*, 765.

Professor Dr. Gerhard Pfleiderer
Institut für Organische Chemie
Biochemie und Isotopenforschung
der Universität Stuttgart
Pfaffenwaldring 55
D-7000 Stuttgart 80